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# Chironomus melanotus Keyl and its taxonomical and ecological relations to C. riihimakiensis Wülker

(Diptera, Chironomidae)

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The karyological, taxonomical and ecological relationships between sympatric populations of *Chironomus melanotus* Keyl and *C. riihimakiensis* Wülker from Riihimäki in southern Finland are described.

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## Introduction

Chironomus riihimakiensis Wülker (1973) was originally found in a small, old clay pond on the Hirvenoja farm, about 5 km north of the town of Riihimäki, in southern Finland. This find first led to a confusion of species when specimens of *C. melanotus* Keyl (1961) were reared from the main ditch, which is flowing through the same farm very near the type locality of *C. riihimakiensis*. *C. melanotus* was identified karyologically by the second author. Possibilities of morphological differences between the species have been sought.

## Karyological studies

Larvae of *Chironomus melanotus* Keyl from Riihimäki, October 1986, November 1987 and June 1988, family Hirvenoja leg., were used. The well known acetorceinic method was applied. The distribution and localization of the constitutive heterochromatin were determined by the "C." banding technique (Michailova 1987). Paratype chromosome preparation of *C. riihimakiensis* from the Zoological Museum, University of Helsinki was used for comparison.

#### Karyotype (N = 11) of *C. melanotus* (Fig. 1)

2n = 8. This species was karyologically described by Keyl (1961) and later by Wülker (1973). *C. melanotus* belongs to the *thummi* complex. The Ist and IInd chromosomes are metacentric, IIIrd submetacentric and IVth acrocentric. Unlike the German population, the Finnish population has large dark heterochromatin blocks not only in the centromere regions of chromosomes AB, EF and G but also in the CD chromosome. The band sequences of arms A, E and F have been described and compared with other *Chironomus* species by Keyl (1962) and Wülker (1973). Arms A, E, F have been divided according to Keyl (1962).



Fig. 1. Chromosomes of *C. melanotus* Keyl; 1st chromosome (AB), 11nd chromosome (CD), 111rd chromosome (EF), 1Vth chromosome (G), nucleolus (N), centromere region (C).

Arm B. Similar to C. *riihimakiensis:* near the centromere region is a light zone followed by two dark bands. Not far from these is a homozygous inversion (in section 26-28) if compared with C. *riihimakiensis.* 

Arm C. In the centre of the arm is a constriction, a typical feature of the *C. plumosus* group (Ryser & al. 1983). Near to this is an active zone, section 5. *C. riihimakiensis* has in the middle of this arm a nucleolus, a region often unpaired (Wülker 1973).

Arm D. This is quite different from that of *C. riihimakiensis* but similar to that of *C. plumosus*. At the telomere region of this arm is a group of dark bands.

Arm G. The homologues are unpaired with heterochromatin in a heterozygous state.

"C" banding (Fig. 2). Heterochromatin bands only in the centromere regions.

This species has no inversion polymorphism in other populations (Keyl 1962, Wülker 1973). The Finnish population has a heterozygous inversion in arm D (20%) (Fig. 3).

The larvae of the present two species can be identified by their karyotypes: sequences of bands in arms A, D, F and G; large heterochromatin blocks present in the centromere regions of *C. melanotus* 



Fig. 2. AB, CD, EF and G chromosomes of C. melanotus Keyl stained by "C" banding.



10 µm

Fig. 3. A heterozygous inversion in IInd chromosome of C. melanotus Keyl.

only. Up to now the latter appeared to be rare in genus *Chironomus:* in *C. crassimanus* (Keyl 1962), *C. cucini* (Martin 1979) and some Swiss populations of *C. plumosus* (Michailova, Fischer 1986) and USSR populations of the same species (Petrova, Kiknadze, Michailova 1985). Perhaps the duplication has played an important role in the formation of the centromere regions. The amount of "C" heterochromatin seems to have increased during evolution. These cytogenetic features allow us to consider *C. melanotus* a derivative species.

Summarizing differences, the species can be distinguished as follows:

# Morphological studies

# Chironomus melanotus Keyl, 1961

Field material from Riihimäki, Hirvenoja, Finland, 1981–1988.



Fig. 4. Male genitalia of C. melanotus Keyl and C. riihimakiensis Wülker.



Fig. 5. Female genitalia and shape of cercus of C. melanotus Keyl.

Male (N = 11)

A blackish species, with a more or less greenish tint. Abdomen unicolorous; thorax dorsally more or less dark. Legs somewhat pale, but femora apically and tibiae proximally clearly darkened and tarsi darkening towards the distal joints.

*AR* 3.78–4.40 (according to Wülker 1973 the lowest value 3.43). Segments of the palps (2-5) 70–90, 240–285, 250–270 and 315–375  $\mu$ m. Clypeus with about 30 setae like vertex and postocular region. Frontal tubercles about 40–50  $\mu$ m. Distance between eyes less than twice breadth of dorsal extension.

Chaetotaxy of thorax and abdomen as usually in this genus; dorsocentral setae in 1-3 rows, 20-35 in number; about 30-45 setae of the scutellum are standing irregularly, but the most caudal setae are nearly in a row. Tracheal scar of pupal respiratory organ ("thoracic sensory pit", Edwards 1929) about one half of the length of postpronotum. Wings 4.3-5.0 mm. Costa not extended over R4+5; setae on R1 and on the distal part of R4+5; squama with about 30 setae.

Sensilla chaetica (about 15  $\mu$ ) near distal end of the basitarsus; in the middle leg 7–14 and hind leg 4–16 in number observed. *P1:LR* 1.43–1.54 (according to Wülker 1973 1.29–1.56), *BR* 2.0–4.4. *P2:LR* 0.59–0.64, *BR* 2.2–4.0. *P3:LR* 0.69–0.74, *BR* 3.9–6.8. Legs (Fe–Ta5) in  $\mu$ m:

 P1
 1205-1640
 1150-1565
 1750-2280
 955-1260
 685-900
 575-825
 260-360

 P2
 1305-1760
 1250-1740
 785-980
 480-640
 335-445
 230-305
 150-215

 P3
 1520-2000
 1550-2085
 1130-1520
 675-955
 480-685
 280-425
 175-250



Fig. 6. *C. melanotus* Keyl; region of chin (hypochilum), mandible, premandible and antenna of the larva, anal comb of the pupa.

Anal tergite of the hypopygium with 7–10 bristles. Characteristic features (Fig. 4) are the moderate broadened (proximally about 65  $\mu$ m) anal point (length/width <3; cf. Lindeberg & Wiederholm 1979, Fig. 1 a) and long appendages 1 (Fig. 1 in Reiss & Fittkau 1971), which are at least 4–5 times longer than broad, usually with a knob-like projection or a fold apically; the styli are more suddenly tapering than in *C. riibimakiensis*.

# Female (N = 9)

The coloration as in male or thorax sometimes light with a greenish touch; abdomen always dark. Chaetotaxy about as in the male, up to 55 dorsocentrals. Antennae with 5 flagellomeres. Segments of the palps (2–5) 70–80, 250–260, 240–265 and 340–365  $\mu$ m. Wings 4.5–5.0 mm; setae as in the male, but some also proximad to *R*4+5, and up to 44 squamal setae.

Sensilla chaetica nearly all the distance along basitarsus, in mid leg 59–79 and hind leg 58–82 in number. *P1:LR* 1.40–1.57, *BR* 1.6–2.3. *P2:LR* 0.53–0.58, *BR* 1.6–2.6. *P3:LR* 0.53–0.58, *BR* 2.4–3.6. Legs in  $\mu$ m:

 P1
 1350-1630
 1260-1520
 1890-2175
 945-1110
 715-825
 675-740
 260-350

 P2
 1435-1715
 1415-1740
 760-945
 415-520
 260-380
 195-250
 175-215

 P3
 1565-1935
 1740-2085
 1175-1455
 655 825
 510-640
 270-325
 175-230

Genitalia as usually in the genus; spermathecae oval,  $140-190 \ \mu m \log$  (Fig. 5).

Pupa (N = 5)

Length of the somewhat pigmented exuvie 8-9 mm. Frontal apotome with about  $100-150 \mu$ m long cephalic tubercles. Thorax dorsally broadly granulated. Shagreen on tergites 2-8 lacking laterally and clearly seen on tergite 7 at the oral half and on tergite 8 at the anal half only as in many other species of this genus. *PB* and about 60-80 intersegmental hooklets on segment 2. *PA* clearly present on the hind corners of segments 4-6 and nearly invisible on the hind corner of segment 7. Dorsal setae on all tergites present. Lateral setae on the abdominal segments 1-8: 0, 3, 3, 3, and filamentous setae 4, 4, 4, (4)5. Anal comb (Fig. 6) of the segment 8 quite large. Anal lobe fringe of filaments multiserial, number of setae 119-141.

# Larva (N = 9)

Length up to 14-15 mm. Claws of anterior parapods apically nearly invisibly serrated. Lateral tubules of segment 10 very short, about 100  $\mu$ m. Two pairs of ventral tubules present, about 1 mm long (Fig. 7). Head,  $650-700 \mu$ m broad (according to Geiger & al. up to 730  $\mu$ m), IC 81-88%. Borders of foramen occipitale dark and head capsule ventrally on the postgenal bridge usually as dark as border of foramen occipitale. Extent of darkened area varies laterally; one pigmented area behind mandibles and pigmented dots on the lateral sklerites of the labrum and antennal sockets. Postgenal bridge light on proximal part of postmentum (Fig. 6), but on the chin or hypochilum (Gouin 1968) where postmentum and postgenal bridge of the head capsule have fused forming the teeth, is dark. Paralabials striated less than a half of their breadth. Distance between the dorsal eyes scen from above 3 times the length of first antennal joint.

Sensory field of labrum with the usual structure of chironomids. Seta  $S_{I}$  (Fig. 7) about 70  $\mu$ m long, quite narrow, apically strongly serrate and the plumose structure on one side only. Setae  $S_{II}$  simple but the 3 long *Ch* setae have weakly plumose sides, clearly to be seen in *Ch* nearest to  $S_{I}$ . Pecten epipharyngis with 12–15 teeth, only one *Chb* seta found, *Chl* setae apically strongly serrate.

Premandibles darkened with 2 apical teeth, inner (dorsal) tooth quite narrow, at least 4 times as long as broad, the outer (ventral) tooth about 2.5 times broader (if seen from apex). Teeth of mandible dark or sometimes the fourth inner tooth more or less light; proximal part of inner side of mandible proximally with few small denticles.



Fig. 7. C. melanotus Keyl and C. riibimakiensis Wülker; top right: last segments and lateral tubules of abdominal segment 7 of the larvae, setae S<sub>1</sub> and below variation of cephalic tubercles of the pupae; left: parts of the head capsules of the larvae dorsal view (melanotus) and ventral view (riibimakiensis).

Median tooth of hypochilum sometimes slightly constricted proximally (as in Fig. 2 in Wülker 1973), but often this cannot be observed; the fifth (in the Russian literature fourth) lateral tooth a little smaller and shorter than the sixth.

Antennae <230  $\mu$ m; *LO* very small, difficult to see; *RO* from base of basal segment in distance about  $\frac{1}{4}-\frac{1}{3}$  the length of this segment; proportions of the segments in the fourth instar:

 $\begin{array}{c} 100:29:10:12:8\\ 100:28:9:11:7\\ 100:27:9:10:7\\ 100:25:9:10:6\\ 100:25:8:10:6\\ 100:22:9:10:6\\ 100:22:9:10:6 \end{array}$ 

### Chironomus riihimakiensis Wülker, 1973

Material. One paratype male, deposited in the Zoological Museum, University of Helsinki. Other specimens, the measured males and females, pupae and larvae from laboratory stock, originally from Riihimäki, Hirvenoja, Finland, reared by M. Hirvenoja in Helsinki, 1970. From this stock eggs were in 1970 sent to professor Wülker (cf. Wülker 1973: 371).

Male (n = 6)

AR 3.25–3.38 (according to Wülker 1973 up to 3.68). Palp segments (2-5) 70–90, 270–290, 270–290 and 350–390  $\mu$ m. Clypeus with about 40–50, vertex and postocular about 20–30 setae. Length of frontal tubercles about 40–50  $\mu$ m.

Thorax and abdomen darkened. Tracheal scar of pupal respiratory organ about one half the length of postpronotum. Chaetotaxy as usually in the genus *Chironomus* with more than 30 dorsocentrals in 1-3 rows. Scutellum with about 30-40 setae from which the most anal are nearly in a row. Wings 4.0-4.4 mm with bristles on R1 and on the apical half of R4+5; costa not lengthened over R4+5. Squama with 18-32 setae.

Number of sensilla chaetica (about 10  $\mu$ m long) 8–15 on mid leg and 5–13 on hind leg, situated near distal end of basitarsus as usual in this genus. *P1:LR* 1.44–1.50 (according to Wülker up to 1.58), *BR* < 2.0. *P2:LR* 0.60–0.66, *LR* 2.0–3.6. *P3:LR* 0.70–0.74, *BR* 2.7–4.7. Legs in  $\mu$ m:

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        P1
        1565-1715
        1480-1665
        2215-2480
        1175-1280
        870-900
        715-760
        335-345

        P2
        1675-1880
        1530-1805
        980-1065
        600-675
        415-445
        280-315
        195-200

        P3
        1870-2045
        1935-2175
        1435-1555
        740-915
        565-650
        350-400
        215-240
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Anal tergite of hypopygium with 6-10 bristles. Characteristic structures (cf. fig. 3 in Wülker 1973) are the moderately broadened anal point (about 70  $\mu$ m, length/width about 3) and the long, quite evenly apically narrowing, and curved first appendages without a clear fold or knob-like projection; first appendage about 3-4 times as long as broad.

Female (n = 8)

Colouration as in male. Antennae with 5 flagellomeres. Segments of palps (2-5) 55–90, 225–270, 240–285 and 315–355  $\mu$ m. Chaetotaxy as in male but number of setae may be higher; dorsocentrals usually 40–60 in number, in 1–4 rows. Wings 4.0–4.4 mm with veins and setae as in the male but, R4+5 with setae also in proximal part.

Observed number of sensilla chaetica 88–118 almost all the length of basitarsus of mid leg and 98–129 in hind leg. *P1:LR* 1.44–1.58, *BR* 1.3–2.0. *P2:LR* 0.57–0.64, *BR* 1.4–2.2. *P3:LR* 0.69–0.72, *BR* 2.3–2.7. Legs in  $\mu$ m:

 P1
 1560-1850
 1415-1685
 2230-2545
 1085-1260
 760-900
 670-780
 320-370

 P2
 1695-1915
 1480-1780
 835-1065
 425-610
 305-435
 175-285
 150-215

 P3
 1825-2065
 1760-2175
 1250-1520
 725 870
 475-650
 280-380
 195-230

Genitalia about as in C. melanotus; spermathecae oval, 130-190 µm long.

Pupa (N = 8)

Length of the quite pale exuvie 8-9 mm. Frontal apotome with about <100  $\mu$ m long cephalic tubercles. Thorax broadly but not very densely or strongly granulated. Shagreen of abdominal tergites 2-8 present, about as in *C. melanotus; PB* and about 80 intersegmental hooklets on second segment. *PA* as in *C. melanotus*. Dorsal setae on all segments present. Lateral setae of segments 1-8: 0, 3, 3, 3 and filamentous setae 4, 4, 4, (4) 5. Anal combs of eighth segment as in *C. melanotus*. Observed number of uni- or biserial laterally arranged filaments of the anal segment 59-77.

Larva (n = 9)

Length up to about 14 mm. Claws of anterior parapods apically nearly invisible serrated. Lateral papillae on tenth segment  $150-250 \ \mu m$  long; two pairs present of weakly strangulated ventral tubules about 2 mm long (Fig. 7). Head  $650-850 \ \mu m$  broad, *IC* 80-85%. Borders of foramen occipitale dark, postgenal bridge, labral sklerites and antennal sockets only weakly darkened.

Sensory field of labrum as in *C. melanotus*, but seta  $S_1$  shorter, about 50  $\mu$ m, plumose all round (Fig. 7);  $S_1$  with hairs seemingly only on one side found, but this depends on mounting position in the slide. All *Ch* setae plumose. Pecten epipharyngis with 13–16 teeth.

Mouthparts typical of the genus. Premandibles with two apical teeth; inner (dorsal) tooth about 3 times as long as broad, outer (ventral) tooth about 2 times broader than the inner (seen from the apex). Mandible with pale proximal (fourth) tooth; inner edge proximally weakly serrated. In hypochilum the unpaired median tooth narrowed proximally as in *C. melanotus*, the fifth and sixth lateral teeth about equal in size; this depends perhaps on orientation on the slide, the fifth tooth may be as short as the sixth.

Antenna <200  $\mu$ m; *RO* from the base of basal segment in distance about 1/4 - 1/3 of the length of this segment; proportions of segments in fourth instar:

100:30:11:11:5 100:28:11:10:5 100:28:10:10:6 100:27:13:11:4 100:26:11:10:6 100:24:11:10:6

#### Ecological comparison

As mentioned, *C. riibimakiensis* was originally found in an old, permanent clay pond ( $2 \times 3$  m, depth < 0.5 m), some meters from a similar but somewhat smaller, ephemeral pond (R2 in Fig. 3 in Hirvenoja 1962: 98). The bottom consisted of clay ooze covered with alder leaves (*Alnus incana* [L.] Willd.) and *Callitriche* vegetation. Conductivity > 8 mS/m, pH about 6 and colour > 80 mg Pt/l depending on the amount of water.

C. riibimakiensis, identified as adult females and males, was collected also by Mr. Petri Nummi in June 1987 in a beaver pond in the small Saukonoja river, (Evo, Lammi), south Finland. The mean depth of the biotope was 0.49 m, velocity of the water 3 - 40 cm/sec, the conductivity 4.4-4.5 mS/m and pH 6.1-6.2 (Nummi, 1989). It thus can live also in a running-water habitat, but is probably less wastewater-tolerant than is C. melanotus.

According to Wülker (1973: 362), *C. melanotus* has been found in humic or eutrophicated ponds. The biotope of *C. melanotus* in Riihimäki is loaded with waste waters from the direction of the town of Riihimäki and with the humic waters of a peat pog. Some features of the monitoring station on 10.V.–26.IX. 1981, when *C. clarus* Hirv. was the dominating species of *Chironomus* in the habitat, were as follows:

width	depth	velocity	temp.	conduct.	pН	color
m	m	cm/s	°C	mS/m (γ25)		Pt mg/1
1.5 - 2.5	0.2 - 1.0	<25	<+22	18.0-45.3	6.6-7.3	140-300

A single water sample on 5.7.1981 gave the following results in mg/1:

P <sub>tot</sub>	$N_{tot}$	К	Ca	Mg	CI	Fe	S	KMn0₄ cons.
0.249	1.71	3.75	10.1	4.1	26.5	0.44	9.9	101.8

#### Taxonomical remarks

The male of *C. melanotus* is very similar to *C. riihimakiensis* on the basis of the structure of the hypopygium or of the morphometrical data.

The key of Lindeberg and Wiederholm (1979), on males, gives the group of *C. aberratus* Keyl, *C. sororius* Wülk., *C. striatus* Str., *C. melanotus* Keyl, *C. lacunarius* Wülk. and *C. riihimakiensis* Wülk. According to the authors, these species are not readily identifiable. The identification of the males of the two present species from reared or trapped samples from the field is possible from the differences in the females and pupae as follows:

- About 90-130 sensilla chaetica on the Ta1 of P2 and P3 of the female. Pupae with abouth 60-70 lateral filaments on anal segment; cephalic tubercles shorter than 100 μm

According to the literature, the size of the abdominal tubules is influenced by the water chemistry of the habitat. We can, however, try to summarize the differences between the species in local populations of the larvae from Riihimäki as follows:

- 2 (1) Lateral tubules of seventh abdominal segment 150-250 μm. Setae S<sub>1</sub> about 50 μm, all round plumose. Borders of foramen occipitale dark, head ventrally weakly darkened. Fourth antennal segment not longer than third. Inner (dorsal) tooth of premandibles about three times as long as proximally broad. Median tooth of hypochilum proximally weakly narrowed and fifth lateral weakly shorter. Ventral tubules of the eighth abdominal segment 2 mm

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